SUPPORTING INFORMATION

Environment-Responsive Alkanol-based Supramolecular Solvents: Characterization and Potential as Restricted Access Property and Mixed-Mode Extractants

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EXPERIMENTAL

Chemicals

All chemicals used were analytical reagent grade and used as supplied. The following reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA): the aliphatic alcohols 1-heptanol (C_7), 1-octanol (C_8), 1-decanol (C_{10}), 1-dodecanol (C_{12}) and 1-tetradecanol (C_{14}); the carcinogenic chlorophenols (CCPs) 2,4,5-trichlorophenol (2,4,5-TCP), 2,4,6-trichlorophenol (2,4,6-TCP), 2,3,4,6-tetrachlorophenol (2,3,4,6-TTCP) and pentachlorophenol (PCP); and the reagents decanoic acid, ochratoxin A (OTA), Brilliant Blue G (BBG), gluten, starch, humic acid sodium salt, Acid Red 97 and chitosan. The carcinogen 2,4-dichlorophenol (2,4-DCP) was supplied by Merck (Darmstadt, Germany). Triton-X114, albumin from bovine serum and chicken egg white, and lysozyme from chicken egg white were obtained from Fluka (Madrid, Spain). Tetrahydrofuran (THF), methanol (MeOH) and LC grade acetonitrile (ACN) were supplied by Panreac (Sevilla, Spain) and ultra-high-quality water was obtained from a Milli-Q water purification system (Millipore, Madrid, Spain). Stock standard solutions containing individual CCPs at a 1 g L^{-1} concentration each were prepared in methanol and stored at 4 ºC in the dark. Working solutions containing a mixture of CCPs were made by appropriate dilution of the stock solutions with methanol.

Phase Diagrams and SUPRAS Volumes

Phase diagrams for the ternary mixture THF:water:*n*-alkanol were constructed in order to delineate the region for SUPRAS formation. Simultaneously, the volume of SUPRAS produced in this region was measured. For this purpose, the three components were mixed at different mass fractions (total weight 10 g) in 10 mL glass centrifuge tubes, magnetically stirred (700 rpm, 1 min) and centrifuged (2500 rpm, 5 min) to accelerate phase separation. Phase boundaries were assigned by visual inspection. The criterion used to determine the formation of SUPRAS was the formation of two immiscible isotropic liquid phases in the system. For alkanols that are liquid at room temperature

(i.e. C_7–C_{10}), the boundary for the amphiphile precipitation and SUPRAS formation regions was established from the difference in volumes between the insolubilized pure alkanol and the SUPRAS, the latter being higher by effect of the incorporation of water and THF —the criterion was an increase by more than 10% in volume. The actual formation of SUPRASs above the boundary was later checked by light microscopy.

The volume of SUPRAS (upper phase) was calculated by measuring its height in the cylindrical tube with a digital calliper. Low SUPRAS volumes (less than ~1 mL) were measured in specially designed 10 mL centrifuge tubes with narrow necks (~7 mm i.d.). All experiments were performed in duplicate.

SUPRAS Composition

The mass fraction of THF, water and *n*-alkanol in the SUPRAS was determined as a function of the composition of the self-assembly environment. Water content was measured with a Karl Fisher coulometric titrator (KF 831model, Methrohm, Herisau, Switzerland). The content in *n*-alkanol was calculated by removing water and THF from a certain amount of SUPRAS (~1 g) and weighing the remaining pure solid or liquid alkanol. For this purpose, SUPRAS aliquots were allowed to stand in open tubes at room temperature for 3 days. This caused all THF and most of the water to evaporate. Then, the SUPRAS was freeze-dried for 24 h to remove any residual water. Finally, the content in THF was calculated as the difference between the total weight and that of the measured components. All experiments were made in triplicate.

SUPRAS Composition and Volume Prediction Equations

Nonlinear regression was used to fit a model to the results (n = 80) in order to predict the SUPRAS volume obtained from a specific alkanol-THF-water ternary mixture in the bulk solution. This procedure uses an iterative approach to minimize the sum-of-squares of the vertical distances for the experimental points to a proposed curve based on preliminary estimates. Equations for predicting SUPRAS composition (THF, water and

n-alkanol content) were defined by fitting experimental data of THF and water content in the SUPRASs as a function of the initial concentration of these components before phase separation to a second-order polynomial regression and a multiple linear regression model, respectively, both based on a least squares approach. The statistical program *Statgraphics Plus (version 3.0)* was used for this purpose.

SUPRAS Structural Characterization

Light microscopy studies were performed with a Leica model DME instrument equipped with an automatic photocamera, using the bright field. As THF easily evaporates under light microscopy observation conditions, only those micrographs taken immediately after each SUPRAS was prepared were considered. Cryo-scanning electron micrographs (Cryo-SEM) were obtained with a CT-1000 cryo-transfer system (Oxford Instruments, Oxford, UK) interfaced to a JEOL JSM-5410 scanning electron microscope. SUPRASs were prepared by dissolving *n*-alkanols (200–500 mg) in a mixture of THF:water (10 mL) in 15mL centrifuge tubes. The mixture was magnetically stirred at 700 rpm for 5 min and then centrifuged (2500 rpm, 5 min) to accelerate phase separation. A drop of SUPRAS was then placed over a piece of 0.2 μm filter paper and frozen in slush N_2, after which it was attached to the specimen holder of the cryo-transfer system for placement on the microscope sample stage, where it was sublimed at a controlled temperature of –90 ºC for 1–3 min —until coacervate droplets appeared. Finally, the sample was again transferred to the cryostage for gold coating by sputtering and then placed back on the microscope sample stage for inspection.

Molecular Size-Based Restricted Access Properties of SUPRASs

Microextraction of OTA from Cereal Baby Food

Aliquots (400 mg) of multicereal based baby food bought at a local supermarket were spiked to a final OTA concentration of 10 μg kg^{-1} and allowed to stand for 12 h before analysis. OTA was microextracted with SUPRASs synthesized from 200 or 300 mg of decanol dissolved in THF:water mixtures (10 mL) in variable ratios (see Table 1). The

sample and SUPRAS were mixed in 2 mL microcentrifuge tubes, vortex agitated (15 min) and centrifuged (15 000 rpm, 10 min). Aliquots of 20 µL of the SUPRAS were then directly analyzed by LC–FL, using a Breeze HPLC system from Waters (Milford, MA), which comprised a 1525 binary pump, a 717 plus automatic injector, a 1500 series column heater, a 2475 multiwavelength fluorescence detector and a 2898 photodiode array detector. The stationary phase was a Kromasil C_8 column (length 25 cm, particle size 5µm, inner diameter 4 mm) from Análisis Vínicos (Tomelloso, Spain). The mobile phase consisted of water (solvent A) and acetonitrile (solvent B), both containing 1% acetic acid. The elution program was as follows: isocratic conditions (55% A) for 5 min, linear gradient from 55% to 50% in A for 15 min and then isocratic conditions (50% A) for the next 15 min. The flow rate was 1 mL min^{-1}. OTA was monitored at λ_{ex} 334 nm and λ_{em} 460 nm. The retention time was 24.0 min.

Microextraction of Dyes from Sludge

Activated and dehydrated sludge were collected from a wastewater treatment plant (WWTP) in Pozoblanco (southern Spain) in December 2010. This WWTP receives household effluents mainly. The dehydrated sludge was freeze-dried in a Telstar Cryodos-50 freeze dryer (Terrassa, Spain), finely ground (<0.5 mm) and stored in glass amber bottles at 4 °C until analysis. Activated sludge was filtered and then processed as dehydrated sludge. Aliquots of the blank sludge sample (300 mg) were spiked to a final concentration of 1.7 mg kg^{-1} with Acid Red 97 and Brilliant Blue G, and allowed to stand for 12 h before analysis. SUPRASs were prepared in the same way as for the OTA determination and extractions done identically. Aliquots of 5 µL of each SUPRAS were analyzed by LC-UV(DAD). The mobile phase consisted of an isocratic mixture of methanol:water (50 mM ammonium acetate) at a flow rate of 1 mL min^{-1}. Acid Red 97 and Brilliant Blue G were determined at 496 and 610 nm, respectively. The retention times were 10.9 and 15.8 min.

Determination of Carcinogenic Chlorophenols in Surface and Ground Waters

Sample Collection and Preservation

Water samples were collected in dark glass containers from two rivers (Guadalquivir and Guadajoz), a reservoir (Navallana), the Mediterranean sea (Los Álamos beach and La Malagueta beach) and the Atlantic sea (Puerto de Santa María beach) in April 2011. All sampling sites were located in Córdoba, Málaga or Cádiz (southern Spain). Samples were filtered through 0.45 μm pore size filters from Whatman GF/F Osmonics in order to remove suspended solids, acidified to pH ~2.5 with 12 M hydrochloric acid and stored at 4 ºC until analysis.

SUPRAS-based Microextraction

1-Decanol (100 mg) was dissolved in 3 mL of THF into 40 mL centrifuge tubes with a narrow neck (7.5 mm i.d.). Then, a sample aliquot of 37 mL (pH ~2.5) was added, which caused the SUPRAS to form instantaneously in a spontaneous manner. The mixture was stirred (1000 rpm, 5 min) to break the solvent up into droplets, which accelerated analyte extraction, and then centrifuged (3500 rpm, 10 min) to speed up separation of the solvent from the aqueous solution. The volume of solvent (~150 μL), which was standing at the top of the solution in the narrow neck of the tube, was calculated by measuring its height with a digital calliper. Aliquots of 100 μL were withdrawn with a microsyringe and transferred to a sealed glass vial with insert (~150 μL capacity) for subsequent analysis.

Liquid Chromatography–Photometry

The liquid chromatographic system used to analyze CCPs consisted of a 1525 binary pump, a 717plus automatic injector, a 1500 series column heater and a 2898 photodiode array detector (Breeze HPLC, Waters, Milford MA). The stationary phase column employed was Ultrabase C_{18} (particle size 5 μm, inner diameter 4.6 mm, length 25 cm) supplied by Análisis Vínicos (Sevilla, Spain), and kept at 32 ºC. The mobile phase consisted of water (solvent A) and methanol/acetonitrile (85:15, solvent B), both

containing 1% acetic acid. The gradient elution program was as follows: isocratic conditions with 63% B for 1 min, linear gradient from 63% to 76% B for 7.5 min and from 76% to 97% B for 4.5 min, then 97% B for 5 min and linear gradient from 97% to 100% B for 2 min. Reconditioning the column took about 10 min. The flow rate was set at 1 mL min^{-1}. The wavelengths used for measurement were 286 nm for 2,4-DCP, 290 nm for 2,4,5-TCP and 2,4,6-TCP, 300 nm for 2,3,4,6-TTCP and 304 nm for PCP. Individual CCPs were quantified by measuring the peak areas provided by 12.5 μL aliquots of SUPRAS containing analyte concentrations over the range 0.05–10 mg L^{-1}.

RESULTS AND DISCUSSION

Equations for SUPRAS composition and volume prediction

The proportion by weight of water in the SUPRAS obtained from a specific alkanol (Y_{ws}) was negatively correlated with both the percentage (w/w) of water in the bulk solution (X_{wb}) and the number of carbon atoms of the alkyl alcohol (Z):

$$Y_{ws} = A - BX_{wb} - CZ \qquad [1]$$

where A, B and C, and their respective standard errors, were 42.2 ± 1.5, 0.31 ± 0.01 and 0.998 ± 0.091, respectively. The correlation coefficient was 0.9729. This relationship indicates that SUPRASs containing high proportions of water can only be obtained from alkanol-THF-water ternary mixtures containing high proportions of THF. On the other hand, the water content in SUPRASs synthesized from a specific alkanol-water-THF mixture will decrease in the following sequence: $C_7 > C_8 > C_{10} > C_{12} > C_{14}$.

The dependence of the THF content of the SUPRAS (Y_{ts} %, w/w) on its content in the bulk solution (X_{tb} %, w/w) was described by the function

$$Y_{ts} = A + BX_{tb} - CX^2_{tb} \qquad [2]$$

where A, B, and C, and their respective standard errors, were 6.3 ± 0.2, 2.4 ± 0.1 and 0.024 ± 0.002. The correlation coefficient was 0.9851. The chain length of the alkanol had no effect on Y_{ts}.

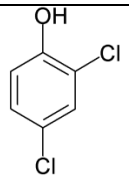
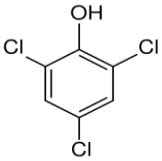
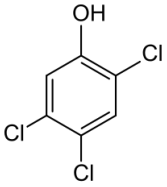
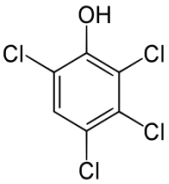
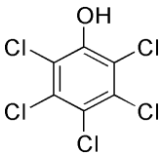
Based on equations 1 and 2, the spontaneous self-assembly process by which alkanol-based SUPRASs form follows predictable routes that can be used to obtain solvents with specific properties. The amount of product obtained (i.e. the resulting solvent volume) can be predicted from the following the equation

$$Y = X\,[A + e^{BZ}] \qquad [3]$$

where the dependent variable, *Y*, is the volume of SUPRAS (μL), while the independent variables X and Z are the amount of alkanol (mg) and the percentage of THF (v/v) in the THF:water mixture in the bulk solution, respectively. Equation 3 provided an estimate of 0.17 ± 0.02 for A and one of 0.0389 ± 0.0003 for B. The correlation coefficient for the equation was 0.986. The linear dependence of the SUPRAS volume on the amount of alkanol further confirmed that the SUPRAS composition was alkanol-independent.

Equations 1–3 were only fulfilled at concentrations of alkanols in the bulk solution lower than around 5% (w/w), which are the typical levels used in analytical applications. Higher concentrations of these amphiphiles gave SUPRASs spanning the composition ranges of Fig. S-1, B; however, THF or water acted as a limiting reactant and neither the composition nor the volume of SUPRAS could thus be predicted. Other variables such as the concentration of sodium chloride and pH only influenced the volume of SUPRAS produced at levels beyond the typical range of analytical interest. Thus, the SUPRAS volume remained constant over the pH range 2–10 and decreased by about 15% at pH 1.5. Regarding salt concentration, SUPRAS volumes were constant up to about 0.1 M and increased around 12% at 1 M.

Table S-1. Structures, octanol:water partition coefficients, acidity constants and estimated carcinogenic potency of various carcinogenic chlorophenols

Compound	Structure	[a]Log K_{ow}	[a]pk_a	[b]IARC carcinogenic potency
2,4-Dichlorophenol (2,4-DCP)		2.9	8.1	2B
2,4,6-Trichlorophenol (2,4,6-TCP)		3.6	6.6	2B
2,4,5-Trichlorophenol (2,4,5-TCP)		3.8	7.1	2B
2,3,4,6-Tetraclhorophenol (2,3,4,6-TTCP)		4.2	5.6	2B
Pentachlorophenol (PCP)		4.8	4.7	2B

[a]Calculated by using Advanced Chemistry Development (ACD/Labs) Software v. 9.04 for Solaris

[b] Possibly carcinogenic to humans according to the *International Agency for Research on Cancer* (IARC)

Table S-2. Mean recoveries and standard deviations (R ± SD, %), and actual concentration factors (ACFs), for carcinogenic chlorophenols as a function of the proportion of THF used to obtain the SUPRAS

	THF (%, v/v)													
	2.5		**5**		**7.5**		**10**		**15**		**20**		**30**	
Carcinogenic chlorophenol	[a]R± [c]SD	ACF	[a]R± [c]SD	ACF	[a]R± [c]SD	ACF	[a]R± [c]SD	ACF	[a]R± [c]SD	ACF	[a]R± [c]SD	ACF	[b]R± [c]SD	ACF
2,4 DCP	80±1	245	80±1	219	88±5	215	92±1	201	88±1	152	83±1	113	47±2	39
2,4,6 TCP	84±2	257	88±3	241	92±3	225	95±2	207	99.2 ±0.4	172	88±1	120	52±2	43
2,4,5 TCP	92±1	282	92±2	252	96±1	235	96±1	210	93±1	161	91±2	124	58±3	48
2,3,4,6 TTCP	92±1	282	92±1	252	95±2	233	99±1	216	92±1	159	88±2	120	59±3	49
PCP	93±1	285	94±2	257	97±2	237	97±1	212	104± 1	180	99±1	135	76±4	63

Spiking levels: [a] 2 µg L^{-1}, [b] 4 µg L^{-1}; [c] n = 3; amount of decanol: 100 mg, volume of THF+water: 40 mL

Table S-3. Mean recoveries and their standard deviations (R ± SD, %), and actual concentration factors (ACF), for carcinogenic chlorophenols as a function of the volume of SUPRAS employed for extraction

Carcinogenic chlorophenol	SUPRAS (volume, µL)											
	61		91		151		302		605		1209	
	[a]R±[c]SD	ACF	[a]R±[c]SD	ACF	[a]R±[c]SD	ACF	[a]R±[c]SD	ACF	[b]R±[c]SD	ACF	[b]R±[c]SD	ACF
2,4 DCP	62±7	379	68±10	277	88±5	215	92±1	113	97±1	59	96±1	29
2,4,6 TCP	66±8	404	78±8	318	92±3	225	94±3	115	98±2	60	97±1	30
2,4,5 TCP	69±5	422	85±8	347	96±1	235	95±4	116	95±2	58	98±2	30
2,3,4,6 TTCP	71±6	434	84±6	343	95±2	233	96±3	117	97±3	59	97±1	30
PCP	65±7	398	81±7	330	97±2	237	97±3	119	97±1	59	101±1	31

Spiking levels: [a] 2 µg L^{-1}, [b] 8 µg L^{-1}; [c] n = 3; proportion of THF: 7.5 % , water sample volume: 37 mL

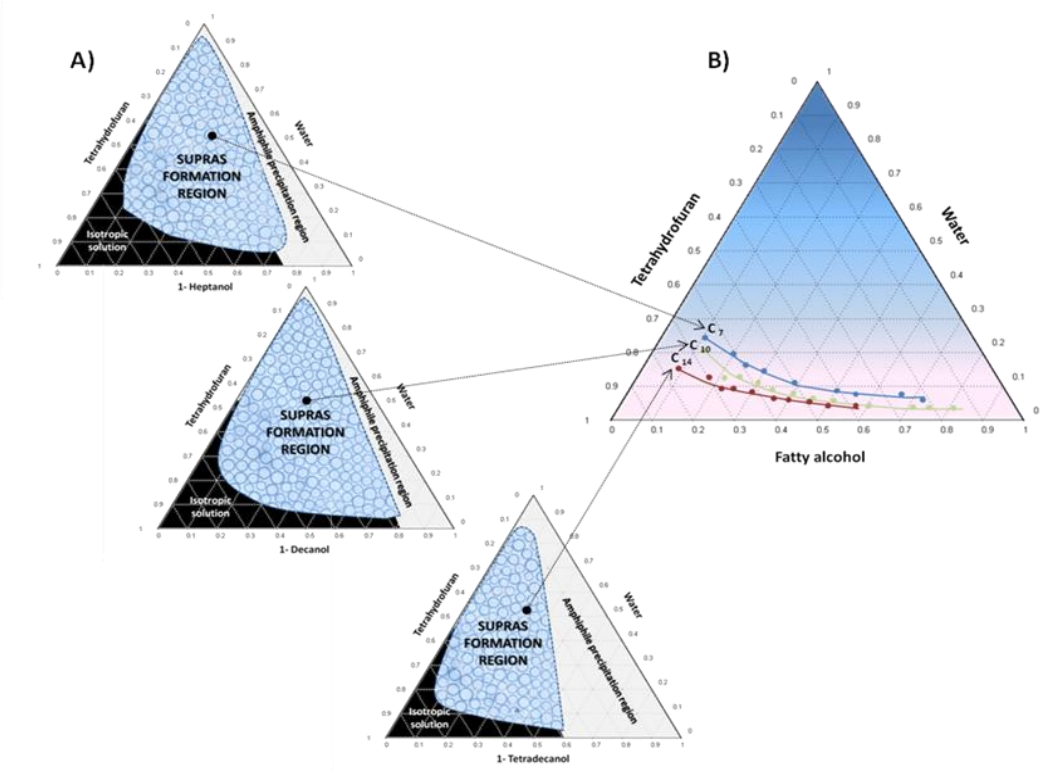


Figure S-1. Phase diagrams showing (A) the behavior of ternary mixtures consisting of alkanol (C_7, C_{10} or C_{14}), THF and water; and (B) the composition of the solvents obtained in the SUPRAS formation region. Concentrations are expressed as mole fractions.

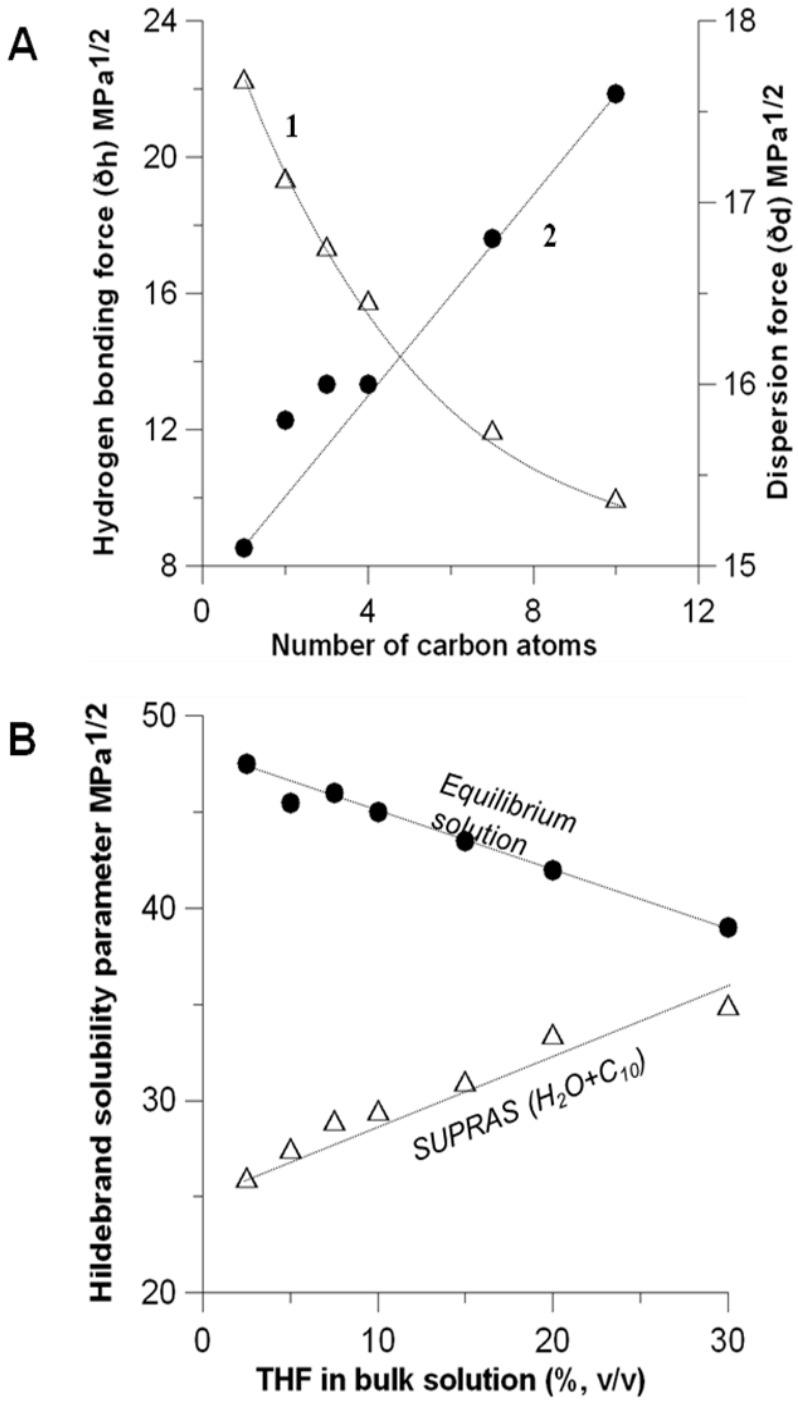


Figure S-2. A) Variation of (1) hydrogen bonding and (2) dispersion forces as a function of the number of carbon atoms in the alkanol and (B) variation of the Hildebrand solubility parameter in the SUPRAS and equilibrium solution as a function of the THF content of the bulk solution.

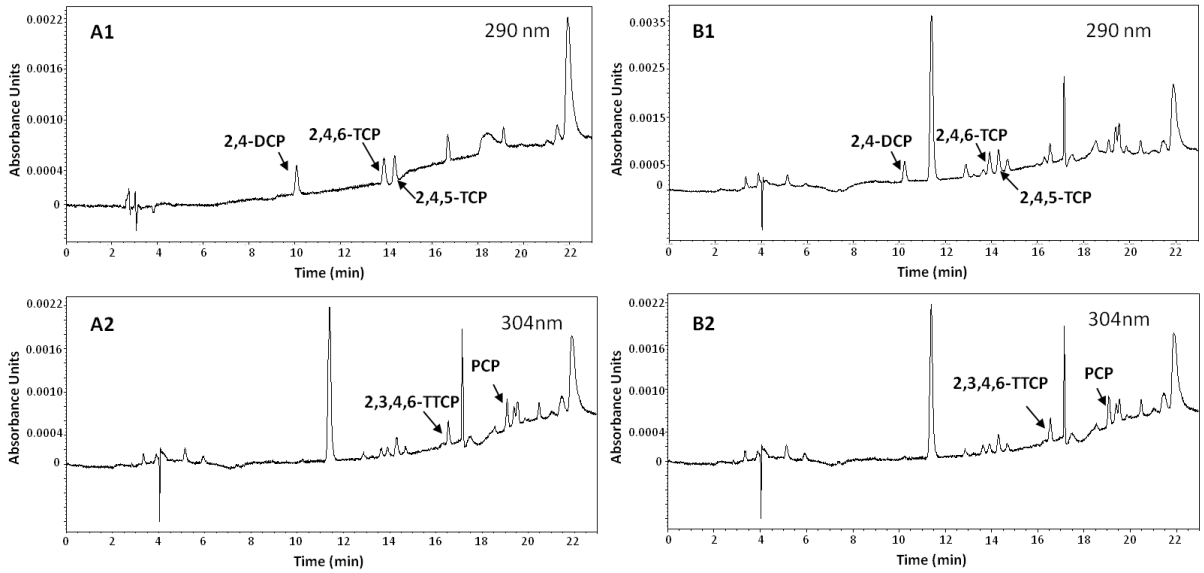


Figure S-3. UV chromatograms obtained from (A) a standard solution of carcinogenic chlorophenols at a 250 μg L^{-1} concentration each in methanol; and (B) a sample collected from "Puerto de Santa María" beach and spiked at 1 μg L^{-1} with CCPs. Wavelengths: (1) 290 and (2) 304 nm.

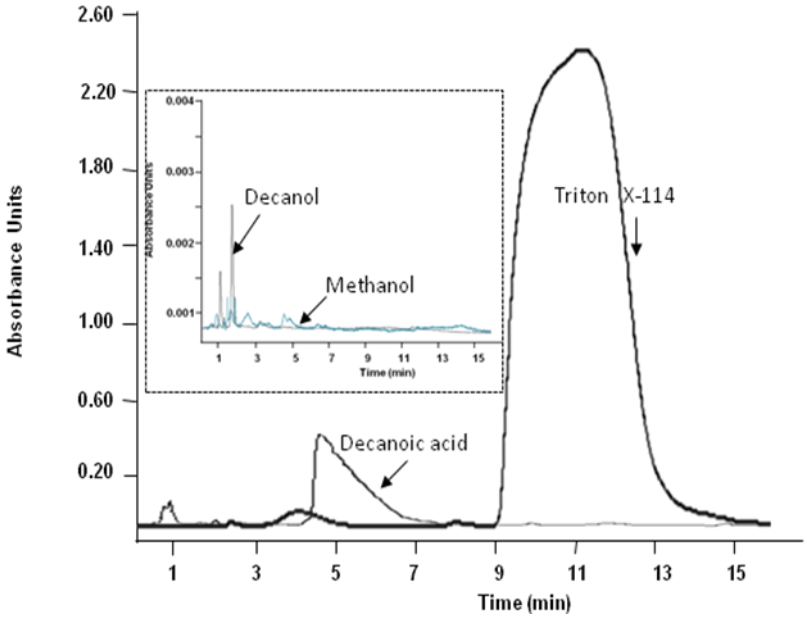


Figure S-4. UV Chromatogram at 230 nm showing background peaks for methanol and SUPRASs consisting of decanol and decanoic acid, both prepared from a percentage of 10% THF (v/v) in the bulk solution and Triton-X114 (5%, w/v in water, room temperature). Chromatographic conditions: Supelcosil LC-PAH analytical column (particle size 3 µm, i.d. 3 mm, length 10 cm); mobile phase consisting of water (A) and acetonitrile (B) with 50% B isocratic conditions for 10 min and then gradient up to 100% B in 1 min; flow rate 0.6 mL min^{-1} ; column temperature 30 ºC; injected volume 3 µL.